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## DOES NITRIFICATION OCCUR IN SEA WATER

DESPITE the meager, observational and experimental data which are available on the subject, the idea of the occurrence and activity of nitrifying bacteria in the open sea is widely prevalent among bacteriologists and botanists. This idea is based, in part, on the reasoning that ocean water should contain the bacteria discharged into it by the sediments and the drainage waters from terrestrial sources; more particularly, however, it is an outgrowth of certain studies which have been made in recent years on the bacterial flora of sea water which, as above indicated, are far from exhaustive and satisfying. For example, Thomsen<sup>1</sup> has discovered nitrite and nitrate producing bacteria in the ooze of the bottom of Kiel Fjord. It has also been reported that nitrite and nitrate forming bacteria have been found in the slime at the bottom of the Bay of Naples. In both cases, however, it is definitely pointed out that the samples studied were obtained from near land surfaces. Moreover, Thomsen failed to discover the nitrite or nitrate forming organisms in sea water or in the plankton or the fixed algae. In commenting on the studies of Keding<sup>2</sup> and Keutner<sup>3</sup> on nitrogen-fixing bacteria of the sea, Drew<sup>4</sup> made the following statement which shows him to have been confused on the subject of two distinct groups of

bacteria wholly different from each other in all respects. He said, "The existence of *nitrifying* bacteria which are *capable of absorbing and combining with the free nitrogen of the air*<sup>5</sup> and eventually give rise to nitrates, has been shown by Keding and Keutner, but these have so far only been found in the bottom close to shore or apparently living in symbiosis with algae or plankton organisms." Later on, however, Issatchenko,<sup>6</sup> whose original papers are not available to me, claims to have found nitrifying bacteria in the Gulf Stream near Ekaterininsk 72° N. He observed, however, that the presence of such bacteria in the Arctic Seas is still unproved. Issatchenko made this statement eight years after having stated, as reported in a brief note,<sup>7</sup> that he had discovered a nitrifying bacterium in Arctic sea water. With these unsatisfactory results before him, Berkeley<sup>8</sup> decided, in the course of other studies on marine bacteria, to make some tests for a possible nitrifying power of sea water. He inoculated 2 per cent. solutions of ammonium sulfate in sea water with samples of the sea water to be studied. He does not state how much inoculum was employed, nor anything else relative to the technique of the experiments, but the result was that even after three months none of the cultures showed even traces of nitrite or nitrate.

In connection with a series of critical studies on the possible connection of bacteria with CaCO<sub>3</sub> precipitation in sea water, which are to appear in the reports of the Department of Marine Biology of the Carnegie Institution of Washington, the writer of this note, unaware of Berkeley's work which appeared at about that time, determined to make some tests for the possible nitrifying power of sea water.

<sup>5</sup> Italics mine.

<sup>6</sup> Issatchenko, B. L.: "Nitrogen Fixation, Nitrification, Denitrification and Production of Hydrogen Sulphide by Bacteria in the Arctic Ocean." *Rev. Agr. Expts.*, Vol. 17, pp. 175-9. Cited in *Bull. Agr. Intelligence*, 7, 1753 (1916).

<sup>7</sup> Issatchenko, B. L. Cited from *Centr. Bakt. etc.*, 2th Abt., No. 13-14, p. 430, 1908.

<sup>8</sup> Berkeley, Cyril: "A Study of Marine Bacteria, Straits of Georgia, B. C." *Trans. Roy. Soc. Can.*, Vol. 13, p. 15.

<sup>1</sup> Thomsen, R.: "Ueber das Vorkommen von Nitrobakterien im Meere," *Wiss. Meeresunters.*, Vol. XI, Kiel.

<sup>2</sup> Keding, M.: "Weitere Untersuchungen über stickstoffbindende Bakterien," *Wiss. Meeresunters.*, Vol. IX, Kiel.

<sup>3</sup> Keutner, J.: "Ueber das Vorkommen und Verbreitung stickstoffbindende Bakterien im Meere," *Ibid.*, Vol. IX, Kiel.

<sup>4</sup> Drew, G. Harold: "On the Precipitation of Calcium Carbonate in the Sea, etc." *Papers from Tortugas Laboratory, Carnegie Inst., Wash.*, Vol. 5, 1914.

The first tests were carried out at Pago Pago in American Samoa, but they were later supplemented by several tests at Berkeley, California, with material collected in Samoa. In the tests just mentioned, the ordinary Omeliansky solutions for nitrite and nitrate formation were employed, and they were inoculated in two series, one with sea water and the other with calcareous sand taken from beneath that same sea water. Twenty-five cc. portions of sea water and about one gram of the calcareous sand were used as inocula. After three weeks, there was no trace of nitrite or nitrate in the sea water inoculation, but very good nitrification in the calcareous sand inoculations. The first result is, therefore, in agreement with that of Berkeley, though reached in ignorance of Berkeley's experiments. It will be noted, however, that Berkeley used sea water media containing 2 per cent. of  $(\text{NH}_4)_2\text{SO}_4$ , which is very different from the weak salt media of the Omeliansky solution which contains only .1 per cent.  $(\text{NH}_4)_2\text{SO}_4$ . In fact, there seems to be no real reason for the use of such high concentrations of ammonium sulfate, and especially in the presence of a concentrated salt solution like sea water. Nevertheless, the agreement in the results of the two tests is noteworthy. Before discussing further the significance of my second result, namely, with the calcareous sand inoculations, it is best to describe some subsequent experiments. Thinking that the period of incubation may have been too short in the sea water cultures above described, since the nitrifying bacteria could not in any case be expected to be present in sea water in great numbers, I repeated the experiments on my return to California from Samoa, and allowed the culture to run for eight months in one case. The results were, however, just the same as in the first series. The inoculations with sea water gave no tests for nitrite or nitrate, and the inoculations with calcareous sand taken directly below that sea water gave marked nitrification.

Even these tests did not entirely satisfy me, however, because I still thought that the number of nitrifying bacteria in sea water might be so small as to render possible their total absence from a 25 cc. sea water inoculum. An

opportunity to make further tests came, however, during my continued studies on marine bacteria and the lime precipitation problem during the past summer (1922), this time at the Tortugas Laboratory of the Carnegie Institution of Washington. On June 9, two 150 cc. portions of Omeliansky's solution were placed in one liter Erlenmeyer flasks and sterilized. When the culture solutions were cool, they were inoculated as follows: one with about ten grams of calcareous sand obtained from the sea bottom near the Loggerhead Key shore; the other with 150 cc. of sea water obtained immediately above the calcareous sand. On June 16, tests were made with Trommsdorff's reagent of the cultures, which were incubated at room temperature (about 27° to 31° C. constantly). No test for nitrite was obtained in either culture. On June 22, the tests were made again. This time, the calcareous sand inoculation showed marked nitrite production, whereas the other culture showed nothing. A further test made on July 21 gave the same results as that on June 22.

All of these tests, taken in conjunction with those of Berkeley and possibly those of Isaatchenko, compel us to the conclusion that either the nitrifying bacteria are absent from the sea water, or they can not function in such concentrated salt solutions. The former alternative is probably the correct one, since it will be remembered that my first tests were with much weaker salt solutions, and as Miss Meek and I have shown in a paper soon to appear in the *Journal of General Physiology*, the nitrifying bacteria can withstand very high salt concentrations. It may, therefore, be concluded with reasonable safety that the nitrifying bacteria are absent from open sea water and that, therefore, no nitrification occurs in such sea water. What may be the state of affairs in small areas of the sea close to land and harboring much organic matter is not directly relevant to this particular inquiry. I hope to secure some information on that point soon.

Not the least interesting feature of my tests on this question, however, is the result obtained with the calcareous sand inoculations. It is remarkable that calcareous sand, which is in constant contact with sea water, should har-

bor vigorous nitrifying organisms, whereas that sea water with which it is in equilibrium in the system should not. It is difficult to account for this, except by assuming that the solution surrounding the sand particles is of a very different nature from that in the sea water above, and yet the possibility of that seems rather remote. Of course small amounts of organic matter covering the sand particles may afford protection for the bacteria. Further experiments which I am conducting may throw some light on this question.

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### A RECENT SCIENTIFIC EXPEDITION TO THE ISLANDS OFF THE WEST COAST OF LOWER CALIFORNIA

At the Berkeley meeting of the Pacific Division of the American Association for the Advancement of Science in 1921 there was appointed a Committee on Conservation of the Marine Life of the Pacific, Dr. Barton Warren Evermann, chairman. One of the first tasks which the committee undertook was the making of recommendations for the protection of certain of the marine mammals or for the gathering of necessary information which would make it possible to advocate a concrete plan in the future. With regard to certain species, there already existed sufficient data so that definite action could be undertaken at once, but with other species practically nothing was known of their present status. This was notably true of the Guadalupe elephant seal, Guadalupe fur seal and southern sea otter, all of which once existed in great abundance along the shores of California and Lower California.

Through the activities of the committee, an expedition was dispatched from San Diego to the islands off the west coast of Lower California on July 9, 1922, for the primary purpose of securing data on the three above mentioned species of mammals. The government of Mexico provided the fisheries patrol boat *Tecate* for the work and met all expenses while the party was in the field. Professor Carlos Cuesta Terron, curator of fishes and reptiles of the National Museum of Mexico, was in charge of the expedition and the Mexican gov-

ernment was further represented by Professor José M<sup>a</sup> Gallegos, of the National Museum, Srs. Joaquin Palacios, inspector, and Rudolfo Lascano, assistant inspector of lighthouses, Sr. Enrique Gonzalez, fisheries inspector, and Sr. Luis Rubio, taxidermist.

Through the intercession of Dr. A. L. Barrows, of the National Research Council, the National Geographic Society rendered financial assistance which made it possible for the committee to enlarge the scope of its work by securing the cooperation of the California Academy of Sciences, represented by Mr. Joseph R. Slevin, assistant curator of herpetology, Mr. Frank Tose, chief taxidermist, and the writer; of the San Diego Society of Natural History, which sent Mr. A. W. Anthony, curator of vertebrates, and Mr. Ernest Hinkley, assistant; and the Scripps Institution for Biological Research, represented by Mr. P. S. Barnhart. Mr. Anthony and the writer were placed in charge of the scientific investigations.

The motor ship *Tecate* was admirably suited to the work in hand and the success of the expedition was in no small measure due to the constant interest of Captain Victor Angulo and his well trained crew. Everything possible was done to aid the observers and collectors during the five weeks in the field.

The expedition returned to San Diego on August 16, after having visited the following islands: Guadalupe, San Martin, Cedros, the San Benitos, Natividad, San Roque, Asuncion, Magdalena and Santa Margarita. Landings were also made at Ensenada, San Quintin Bay, San Bartoleme Bay and Abreojos Point on the Lower California peninsula. Besides making collections at all of these places the coast line was studied at close range for considerable distances from the vessel, particularly the bight known as San Cristobal Bay, where elephant seals are known to have once hauled out in numbers.

The herd of elephant seals on Guadalupe Island was carefully studied and counted and, although the results can not as yet be announced, it may be stated that conditions were very encouraging for the perpetuation of this remarkable species. Many interesting photographs, including motion pictures, were taken of the animals.